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Elevation of IgA anti-epidermal transglutaminase antibodies in dermatitis herpetiformis

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Summary

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Conflicts of interest
None declared.

Background Dermatitis herpetiformis (DH) is a papulovesicular eruption caused by ingestion of gluten. It is characterized by the deposition of IgA in the dermal papillae. IgA antibodies directed at tissue transglutaminase (TG2) are elevated in gluten-sensitive diseases including DH and coeliac disease (CD). More recently, antibodies directed at epidermal transglutaminase (TG3) were identified in patients with DH, and this may be the dominant autoantigen in this disease.
Objectives To measure IgA antibodies to TG3 and TG2 in patients with DH and CD, and control populations.
Methods Serum IgA antibodies against TG2 and TG3 were measured from adults with DH, adults and children with CD, patients with psoriasis, adult Red Cross blood donors, and paediatric controls.
Results Patients with DH and CD had elevated levels of IgA anti-TG2 antibodies compared with control populations. The levels in the patients with DH and adults with CD were similar. IgA anti-TG2 antibodies were higher in the children with CD compared with adults with DH and CD, and with control populations. Patients with DH and adults with CD had elevated levels of IgA anti-TG3 antibodies compared with children with CD and control populations. There was a trend towards higher levels in the patients with DH compared with adults with CD.
Conclusions IgA antibodies to TG3 are elevated in patients with DH and adults with CD. The progressive expansion of the epitope-binding profile of IgA antitransglutaminase antibodies in patients with CD may explain the development of DH in patients with undiagnosed CD during their adult life.

Dermatitis herpetiformis (DH) is a papulovesicular cutaneous eruption precipitated by ingestion of gluten.[1] Patients typically present with pruritic, excoriated papules on extensor surfaces of the elbows, knees, buttocks, back and scalp. This eruption is related to IgA deposits in the dermal papillae, a finding that is pathognomonic for DH.[2] These granular deposits of IgA are present in perilesional as well as uninvolved skin. The concentration of granular IgA at these sites decreases with adherence to a gluten-free diet, and reaccumulation occurs with the institution of a gluten-containing diet.[1] The antigenic specificity of the IgA in DH skin is unknown. In 2002 Sardy *et al.*[3] demonstrated the presence of epidermal transglutaminase (TG3) antigen within DH tissue. They proposed that this was the 'dermatitis herpetiformis autoantigen'. However, its relationship to disease activity and the mechanism by which it may participate in the production of the inflammatory process remain unclear. We recently produced a new anti-TG3 antibody and confirmed the observation of Sardy *et al.* that TG3 colocalizes with IgA in DH skin. TG3 was present in both inflamed, involved skin as well as uninvolved skin of patients with DH.[4] This indicates that the presence of the TG3 antigen is not the essential factor in disease pathogenesis. Therefore, TG3 is likely to play an important role in the pathogenesis of DH, but its exact role remains unknown.

Transglutaminases are a family of enzymes with pleiotropic functions, including protein crosslinking, deamidation, amine incorporation, esterification and hydrolysis.[5] There

are nine known human transglutaminases, and some are expressed in the epidermis. The functions of transglutaminases are important in multiple systems, including blood clotting, apoptosis and the maturation of the keratinocyte cytoskeleton. Genetic deficiency of TG2 causes lamellar ichthyosis, and other transglutaminases are also associated with genetic disease.[6]

DH is known to be associated with gluten-sensitive enteropathy (GSE) in virtually all cases.[7] However, the intestinal inflammatory process in DH is generally less severe than that seen in symptomatic coeliac disease (CD). Tissue transglutaminase (TG2), the antigen of an immune response in CD, is widely expressed in many tissues, including the gut epithelium.[5] The mechanism by which an immune response against TG2 is induced by gluten is unclear. However, gluten and gliadin proteins are glutamine rich and contain runs of polyglutamine. Glutens are also substrates of transglutaminase and it is hypothesized that a neoantigen is created by enzymatic modification of dietary gluten.[8] TG3 has a more restricted tissue expression. It also has low sequence homology with other transglutaminases and a relatively low conservation between species.[9] The trigger of the immune response to TG3 in the progression from CD to DH is unclear. However, it is likely that the disease-related antibodies are directed at post-translational epitopes.

The age at onset of CD is reported to be predominantly in early childhood with 70% of cases occurring before the age of 2 years,[10] although with increased sensitivity of serological assays more cases are now being diagnosed in adulthood. In contrast, the mean age at onset of DH is 38 years and childhood cases are very rare.[11]

We have hypothesized that IgA antibodies to TG3 are related to the inflammatory process, disease activity or development of inflammation over a period of time in patients with DH. To test this hypothesis we measured TG3 and TG2 IgA antibody levels by enzyme-linked immunosorbent assay (ELISA) in patients with DH, adults and children with CD and control populations including patients with psoriasis, Red Cross blood donors and paediatric controls.

Materials and methods

Study population

These studies were approved by the Institutional Review Board at the University of Utah. The patient groups in this study included 44 patients with newly diagnosed DH. These patients were a cross-section of our patients with clinical DH. To be considered eligible for the study, patients required a diagnosis of DH established by skin biopsy using direct immunofluorescence showing granular IgA deposits in dermal papillary tips. The age range for the patients with DH was 19–80 years. The first control group consisted of patients with psoriasis not known to have DH. This included 37 patients. The age range was not specified, but all were greater than 18 years. The second control group included 53 randomly ascertained Red Cross blood donors whose samples were collected without knowledge of any clinical symptoms. The age range was not specified, but all were greater than 18 years. The third control group included 50 randomly ascertained children from ARUP laboratories (Salt Lake City, UT, U.S.A.) aged 7–17 years without known DH or CD. Finally, we included 19 adults with new-onset CD and 16 children with new-onset CD. To be considered eligible for inclusion in the study, patients required a small bowel biopsy demonstrating evidence of CD. The duration and severity of clinical symptoms were unknown. The age range of the adults with CD was 20–79 years and that of the children with CD was 1–17 years.

All of the patients with DH and the adults and children with CD were on a normal, gluten-containing diet. No patients with DH were being treated with dapsone at the time of serum collection. None of the patients with DH, adults with CD or children with CD had partial or total IgA deficiency.

Tissue transglutaminase and epidermal transglutaminase assays

Serum IgA antibodies against TG2 and TG3 were measured using a commercially available ELISA assay. Serum was obtained from whole blood by centrifugation for 10 min and frozen at −80 °C with sodium azide. Semiquantitative detection of IgA anti-TG2 antibodies was performed per manufacturer protocol using the QUANTA Lite human recombinant TG2 IgA kits (INOVA Diagnostics, San Diego, CA, U.S.A.). Per manufacturer recommendations, positivity was assigned at ≥ 20 units. Semiquantitative detection of IgA antihuman recombinant TG3 was performed per manufacturer protocol using IgA anti-TG3 ELISA kits (Immundiagnostik, Bensheim, Germany). The upper limit of normal for this assay is assigned as 18 units. Assays were run in duplicate for all sera.

Statistical analysis

A univariate analysis of variance (ANOVA) was used to test the differences in mean IgA anti-TG2/TG3 values among the six groups (adult CD, DH, paediatric CD, psoriasis, Red Cross blood donor and paediatric control). Tukey's multiple comparison adjustment was further performed to determine which groups showed differences if an overall ANOVA was significant. In cases where data were skewed and did not have equal variance across groups, the response variables were then log-transformed and ANOVA was used on the transformed data. A nonparametric Kruskal–Wallis test was utilized to test the differences in IgA anti-TG2/TG3 among groups.

Results

Tissue transglutaminase

Patients with DH and CD had elevated levels of IgA anti-TG2 (Fig. 1). Twenty of 44 patients with DH (45%) had IgA

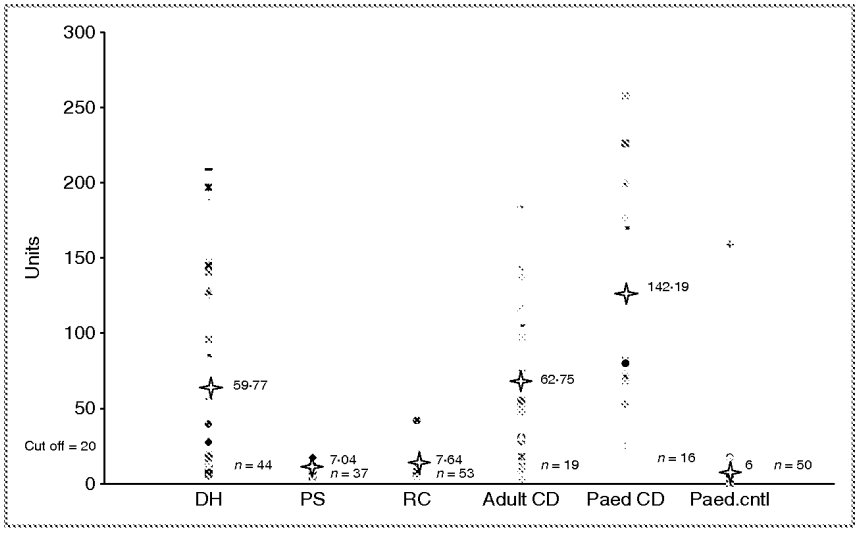

Fig 1. Tissue transglutaminase (TG2) values in 44 patients with dermatitis herpetiformis (DH), 37 patients with psoriasis (PS), 53 Red Cross blood donors (RC), 19 adults with coeliac disease (CD), 16 children with CD (Paed CD) and 50 paediatric controls (Paed.cntl). All patients were on normal, gluten-containing diets. Patients with DH and adults with CD had elevated levels of IgA anti-TG2 compared with Red Cross blood donors, patients with psoriasis and paediatric controls. Children with CD had higher levels of IgA anti-TG2 than both patients with DH and adults with CD. IgA anti-TG2 levels in adults with CD and patients with DH were similar. Mean values are indicated by a cross.

anti-TG2 levels > 20 units. The mean of our 44 patients was 59·77 (range 6–209). Likewise, 15 of 19 adults with CD (79%) had an elevation of IgA anti-TG2 with a mean of 62·75 (range 3–184). The children with CD had higher levels of IgA anti-TG2 (mean 142·19, range 26–258). All (n = 16) of these patients had elevated IgA anti-TG2 levels. In contrast, the group of patients with psoriasis had a mean level of 7·04 (range 5–7) comparable with the Red Cross blood donors (mean 7·64, range 5–42) and the paediatric control group (mean 6·0, range 0–159). There was one Red Cross blood donor and one paediatric control with an elevated IgA anti-TG2 level.

For statistical analysis, a univariate ANOVA was used to test the differences in mean IgA anti-TG2 values among groups. The differences in means in the six groups were highly significant ($P < 0·001$). Homogeneous subsets included (i) patients with psoriasis, Red Cross blood donors and paediatric controls; (ii) adult CD and adult DH; and (iii) paediatric CD, with no significant differences within a subset, and significant differences between subsets. Similar results were found when values were log-transformed and ANOVA used on the transformed data. Finally, a nonparametric Kruskal–Wallis test was performed to analyse the differences among values (not the means) among groups. There were significant differences among homogeneous groups (i, ii, iii) ($P < 0·001$).

Epidermal transglutaminase

Patients with DH had elevated levels of IgA anti-TG3 (Fig. 2). Twenty-three of 44 (52%) had IgA anti-TG3 levels > 18 units. The mean of our patients was 30·73 (range 7–153). Adults with CD also had elevated levels. Ten of 19 (53%) had elevated levels (mean 18·95, range 6·3–58). Children with CD did not have elevation of TG3. Only one of 16 (6%) had an elevated level (mean 9·02, range 3·92–21·14). The psoriatic, Red Cross blood donor and paediatric control populations had means of 6·23, 7·87 and 2·0, respectively. This was within the normal range. A single Red Cross blood donor had an elevated IgA anti-TG3 level of 20.

A univariate ANOVA was used to test the differences in mean IgA anti-TG3 values among groups. The differences among the groups were highly significant ($P < 0·001$). Homogeneous subsets were (i) psoriasis, Red Cross blood donors, paediatric controls and paediatric CD; and (ii) adult CD and adult DH, with no significant differences among subsets, and significant differences between subsets. When a Tukey's multiple comparison adjustment was made, the difference between adult CD and DH was close to statistical significance ($P = 0·053$). Similar results were seen on log-transformed data using ANOVA. A nonparametric Kruskal–Wallis test was used to analyse the differences in IgA anti-TG3 values (not means) among groups and showed significant differences among homogeneous groups (i, ii) ($P < 0·001$).

In summary, patients with DH and CD had comparable levels of IgA anti-TG2 antibodies. However, patients with DH had higher levels of IgA anti-TG3 antibodies, nearing traditional statistical significance ($P = 0·053$).

Discussion

We have evaluated the role of serum IgA antibody assays to TG2 and TG3 in patients with DH, adults and children with CD, and control populations. Perhaps most interesting is the fact that our children with CD had significantly lower levels of serum IgA anti-TG3 antibodies than our adults with CD. This occurred in the face of significantly elevated IgA anti-TG2 levels in the paediatric population compared with the adult population. There is an obvious dichotomy in the expression of the two antibody profiles in these age-controlled groups of patients with the same disorder. We find this particularly interesting because the average age at onset of DH in our patient population is 38 years.[11] The reason for the adult age at onset of DH compared with the frequent childhood onset of CD is unknown. The current data suggest to us that during childhood, patients with CD initially have elevated levels of

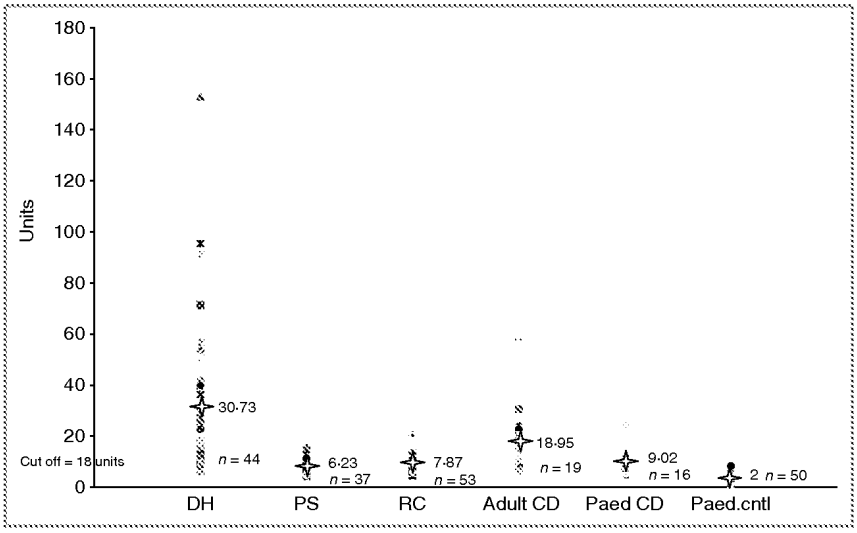

Fig 2. Epidermal transglutaminase (TG3) values in 44 patients with dermatitis herpetiformis (DH), 37 patients with psoriasis (PS), 53 Red Cross blood donors (RC), 19 adults with coeliac disease (CD), 16 children with CD (Paed CD) and 50 paediatric controls (Paed.cntl). All patients were on normal, gluten-containing diets. Patients with DH had elevated levels of IgA anti-TG3 compared with all other groups. Adults with CD had higher levels of IgA anti-TG3 than children with CD. There was a trend towards higher levels in patients with DH compared with adults with CD, but the results did not reach statistical significance ($P = 0{\cdot}053$). Mean values are indicated by a cross.

IgA anti-TG2 antibodies but normal levels of IgA anti-TG3 antibodies. Although we do not have longitudinal studies of any individual patients, the data suggest to us that as time goes on and patients with untreated CD become adults, they develop elevated levels of IgA anti-TG3 antibodies compared with their childhood counterparts. We would propose that this group of patients with CD who develop elevated levels of IgA anti-TG3 antibodies over time comprises the patients at risk of developing DH in adulthood.

We have demonstrated that patients with DH have elevated levels of IgA anti-TG3 antibodies compared with control Red Cross blood donors. This has been described previously by Sardy *et al.*[3] However, Sardy *et al.* failed to note that the serum IgA anti-TG3 levels of patients with DH were higher than those of their control CD population. We tested 44 adults with DH for IgA anti-TG3 antibodies. Adults with DH and adults with CD both had elevated levels of IgA anti-TG3 antibodies compared with control groups and children with CD. There was a trend toward elevated levels of anti-TG3 antibodies in adults with DH compared with adults with CD although this did not quite reach statistical significance ($P = 0{\cdot}053$). Perhaps a larger sample size would demonstrate significance.

In our study, approximately 50% of patients with DH were positive for IgA anti-TG3 antibodies. This suggests that factors other than IgA anti-TG3 antibodies are important in the disease pathogenesis of DH. It is known that IgA is present in DH skin for years and does not correlate with disease activity.[1] Circulating TG3 antibodies may fluctuate with gluten intake, intestinal inflammation or trauma to skin, and may be an indication of disease activity only at the time of serum collection. Further studies to correlate IgA anti-TG3 antibodies in patients with DH with disease parameters including disease duration, gluten intake, disease severity and dapsone dose are warranted to investigate this issue further.

In the TG2 studies we noted that both adults with DH and adults with CD had comparable levels of IgA anti-TG2 antibodies. This is similar to previously reported studies.[12,13] Interestingly, our paediatric CD population had higher levels of IgA anti-TG2 antibodies ($P < 0{\cdot}001$) compared with adults with DH and CD. As expected, the serum IgA anti-TG2 levels of patients with psoriasis, Red Cross blood donors and paediatric controls were within the normal range. Rare patients with psoriasis have been said to have CD causally related to their psoriasis but this apparently did not influence our data.[14,15] These data provide further support for the evaluation of IgA anti-TG2 antibodies in patients with DH and CD and indicate that children with CD may have higher levels of these antibodies than their adult counterparts as well as adults with DH. These results warrant additional, larger studies in adults and children with CD specifically addressing any differences in IgA anti-TG2 or TG3 antibody levels, and any correlation of the antibody levels to severity of disease, duration of disease, or level of gluten restriction.

In summary, our data show the first evidence that progressive expansion of the epitope-binding profile of IgA antitransglutaminase antibodies may be the reason for development of DH in patients with undiagnosed GSE during their adult life.

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References

1 Leonard J, Haffenden G, Tucker W *et al.* Gluten challenge in dermatitis herpetiformis. *N Engl J Med* 1983; **308**:816–19.
2 Zone JJ, Meyer LJ, Petersen MJ. Deposition of granular IgA relative to clinical lesions in dermatitis herpetiformis. *Arch Dermatol* 1996; **132**:912–18.
3 Sardy M, Karpati S, Merkl B *et al.* Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. *J Exp Med* 2002; **195**:747–57.
4 Donaldson MR, Zone JJ, Schmidt LA *et al.* Epidermal transglutaminase deposits in perilesional and uninvolved skin in patients with dermatitis herpetiformis. *J Invest Dermatol* 2007; **127**:1268–71.

5 Kim SY, Jeitner TM, Steinert PM. Transglutaminases in disease. *Neurochem Int* 2002; **40**:85–103.
6 Eckert RL, Sturniolo MT, Broome AM *et al.* Transglutaminases in epidermis. *Prog Exp Tumor Res* 2005; **38**:115–24.
7 Katz SI, Strober W. The pathogenesis of dermatitis herpetiformis. *J Invest Dermatol* 1978; **70**:63–75.
8 Sjöström H, Lundin KE, Molberg Ø *et al.* Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. *Scand J Immunol* 1998; **48**:111–15.
9 Kim IG, Gorman JJ, Park SC *et al.* The deduced sequence of the novel protransglutaminase E (TGase3) of human and mouse. *J Biol Chem* 1993; **268**:12682–90.
10 Bottaro G, Failla P, Rotolo N *et al.* Changes in coeliac disease behaviour over the years. *Acta Paediatr* 1993; **82**:566–8.
11 Smith JB, Tulloch JE, Meyer LJ *et al.* The incidence and prevalence of dermatitis herpetiformis in Utah. *Arch Dermatol* 1992; **128**:1608–10.
12 Dieterich W, Laag E, Bruckner-Tuderman L *et al.* Antibodies to tissue transglutaminase as serologic markers in patients with dermatitis herpetiformis. *J Invest Dermatol* 1999; **113**:133–6.
13 Koop I, Ilchmann R, Izzi L *et al.* Detection of autoantibodies against tissue transglutaminase in patients with celiac disease and dermatitis herpetiformis. *Am J Gastroenterol* 2000; **95**:2009–14.
14 Michaelsson G, Gerden B, Hagforsen E *et al.* Psoriasis patients with antibodies to gliadin can be improved by a gluten-free diet. *Br J Dermatol* 2000; **142**:44–51.
15 Woo WK, McMillan SA, Watson RG *et al.* Coeliac disease-associated antibodies correlate with psoriasis activity. *Br J Dermatol* 2004; **151**:891–4.

